KOZO NISHIO*: Investigation of Pteridophyta plants with the Mäule test

西尾康三*: シダ植物のモイレ反応

This work is an observation of Pteridophyta plants in application of the Mäule test for the purpose of throwing some light on the study of evolution of this group of plants. A color reaction based upon a pretreatment with chlorine followed by a weak base is known as "the Mäule test for lignin". The fact that red color (positive) is shown by angiosperms and yellow color (negative) by both gymnosperms and pteridophytes has been stated by several researchers and this has been accepted as a general rule. However, some exceptions were found in pteridophytes as well as gymnosperms, showing positive colors with this test. Mäule (1902)1), the founder of the test, obtained a positive color in *Selaginella caesia*. Siersch (1926)2) also obtained a result that 4 out of 6 species of *Selaginella* gave a bright red color, while the other vascular cryptogams showed negative colors. Gibbs (1957)3) made a comprehensive review of works supplemented with the result of his own work, and in pteridophytes he stated about noticeable positive colors only in the genus *Selaginella*. The author (1961)4) (1962)5) tested 446 species of vascular plants, including 49 species of pteridophytes among them. He found that 3 species of *Selaginella* and 6 species of Filicales were positive in the reaction, while the other species of pteridophytes were negative. The present investigation is planned to extend this research in the pteridophytes.

Materials and methods Plants used. 27 families, 97 genera, 205 species.

Preparation of samples. Samples to be observed were mostly taken from petioles of plants. Stems were observed when petioles were difficult to use (e. g. Lycophyta plants). The samples were observed in living states with a few exceptions which were observed in herbarium materials in cases when living samples were not available. The samples to be observed were prepared by making hand sections. The result of the Mäule test was observed in the section on the cells which had been known as lignified by the reaction with phloroglucinol and hydrochloric acid on sections as similar as possible.

Reagent, treatment and reaction. Phloroglucinol and hydrochloric acid (Wiesner's reagent). Dissolve 3% phloroglucinol in 100 cc 12% aqueous solution

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of hydrochloric acid. This reagent gives a purple red color with lignin.

Mäule test. Dip a section into 1% aqueous solution of potassium permanganate for 3 minutes. Wash in water for 3 minutes. Repeat this wash. Dip a section into 12% aqueous solution of hydrochloric acid for 3 minutes. Wash in water for 6 minutes. Add 25% aqueous solution of ammonium hydroxide. In this research a purple red color and similar colors are noted as distinctly positive reactions; those of red, orangish red, orange red and reddish orange as indistinctly positive reactions and those of orange, reddish brown, brown orange yellow, brownish yellow as negative reactions.

Results The results are shown in Tab. 1. The way of designating colors in the reaction in what the author recommended in the preceding paper⁽⁶⁾. Sign + in Tab. 1 indicates that some distinctly positive cells in the Mäule reaction are found in any section prepared from plants of the species referred. Sign (+) indicates that some indistinctly positive cells are found. Sign (+) – indicates that some indistinctly positive cells are found in a section prepared from a plant which belongs to the species referred, while only Mäule negative cells are found in a section prepared from another plant which belongs to the same species. Sign – indicates that only Mäule negative cells are found in each section prepared from plants of the species referred. Sign ♯ shows that the reactions are observed with samples from herbarium specimens. Scientific names and the taxonomical system of plants used in the present investigation are referred from "Coloured Illustration of the Japanese Pteridophyta" by M. Tagawa (1959)[7) as far as those are contained in it. As for plants, the names of which are not contained in this book, they are supplemented from "Genera Filicum" by E.B. Copeland (1947)[8).

The table is not shown at the level of tissue. In Pteridophyta lignified cells are generally found in the epidermis, cortex and xylem. No cells which show distinctly positive colors with the Mäule test are found in the xylem even if positive colored cells are found in the other tissues observed in the section. Among 97 genera of Pteridophyta tested, *Selaginella* (6), *Monachosorum* (2), *Ceratopteris* (2), *Elaphoglossum* (2) and *Colysis* (4) showed some distinctly positive reaction in every species tested. The number in ( ) designates the number of species observed in each genus. In *Plagiogyria*, *P. euphlebia* showed distinctly positive and *P. japonica* indistinctly positive, while *P. adnata*, *P. matsumureana* and *P. stenoptera* showed negative. In *Dennstaedtia*, *D. hirsuta* showed distinctly positive, while *D. scabra* showed negative. In *Pteridium aquilinum*, the majority of plants tested showed negative reaction, but the rest showed some indistinctly positive cells among 13 plants tested⁶⁾. In *Lycopodium squarrosum*, two plants were tested. One showed indistinctly positive reaction and the other negative reaction. All the other genera showed negative reaction in the Mäule test. This result would make some contribution to the study of phylogeny of Pteridophyta.

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Tab. 1. Results of the test. (Signs explained in the text.)

Psilotaceae	
Psilotum nudum	−
Lycopodiaceae	
Lycopodium chinense	−
L. serratum	−
L. squarrosum	(+) −
L. fordii	−
L. fargesii	−
L. sieboldii	−
L. phlegmaria	−
L. clavatum	−
L. complanatum	−
L. casuarinoides	−
L. cernuum	−
Selaginellaceae	
Selaginella nipponica	+
S. remotifolia	+
S. uncinata	+
S. doederleinii	+
S. involvens	+
S. tamariscina	+
S. heterostachys	+
Isoetaceae	
Isoetes japonica	−
Equisetaceae	
Equisetum arvense	−
E. limosum	− #
E. hyemale	−
E. ramosissimum	−
Botrychiaceae	
Sceptridium ternatum	−
S. japonicum	−
Japonobotrychium virginianum	−
Ophioglossaceae	
Ophioglossum vulgatum	− #
O. petiolatum	− #
Helminthostachys zeylanica	−
Angiopteridaceae	
Angiopteris lygodiifolia	−
Osmundaceae	
Osmunda japonica	−
O. lancea	−
Osmundastrum cinnamomeum	−
Plenasium banksiaefolium	−
Schizaeaceae	
Schizaea pennula	− #
S. incurvata	− #
Lygodium japonicum	−
Gleicheniaceae	
Dicranopteris dichotoma	−
Gleichenia japonica	−
Hymenophyllaceae	
Mecodium polyanthos	−
M. badium	−
Hymenophyllum barbatum	−
Selenodesmium obscurum	− #
Crepidomanes makinoi	−
Gonocormus minutus	−
Vandenboschia radicans	−
Callistopteris apiifolia	− #
Pteridaceae	
Cibotium balometz	−
Dennstaedtia scabra	−
D. hirsuta	+
Microlepia marginata	−
M. pseudo-strigosa	−
M. strigosa	−

M. lingusta	—
Monachosorum flagellare	+
M. arakii	+
Ptilopteris maximowiczii	—
Lindsaea cultrata	—
L. chienii	—
Sphenomeris chusana	—
S. biflora	—
Hypolepis punctata	—
Pteridium aquilinum	(+) —
Histiopteris incisa	— #
Pteris multifida	—
P. ryukyuensis	—
P. inaequalis	—
P. kiuschiuensis	—
P. oshimensis	—
P. natiensis	—
P. wallichiana	—
Coniogramme intermedia	—
Cheilanthes chusana	—
C. kuhnii	—
Onychium japonicum	—
Pellaea rotundifolia	—
Pleurosoriopsis makinoi	—
Adiantum edgeworthii	—
A. capillus-veneris	—
A. monochlamys	—
A. pedatum	—
Parkeriaceae	
Ceratopteris thalictroides	+
C. pteridoides	+
Davalliaceae	
Davallia mariesii	—
D. solida	—
Humata repens	—
Nephrolepis auriculata	—
N. biserrata	—
Plagiogyriaceae	
Plagiogyria adnata	—
P. japonica	(+)
P. euphlebia	+
P. matsumureana	—
P. stenoptera	—
Cyatheaceae	
Cyathea fauriei	—
C. podophylla	—
Aspidiaceae	
Matteuccia struthiopteris	—
M. orientalis	—
Onoclea sensibilis	—
Woodsia polystichoides	—
Polystichum tripteron	—
P. polyblepharum	—
Cyrtomium falcatum	—
C. fortunei	—
Polystichopsis pseudo-aristata	—
P. amabilis	—
P. mutica	—
P. standishii	—
P. miqueliana	—
Bolbitis subcordata	—
B. laxilaticulata	—
Elaphoglossum yoshinagae	+
E. tosaense	+
Dryopteris sieboldii	—
D. crassirhizoma	—
D. uniformis	—
D. mayebarae	—
D. sabaei	—
D. varia	—
D. sacrosancta	—
D. championii	—
D. hondoensis	—
D. gymnosora	—
Ctenitis maximowicziana	—
C. subglandulosa	—
C. eatonii	—
Ctenitopsis sinii	—
Tectaria devexa	—
T. fauriei	—
Phegopteris decursive-pinnata	—
P. polypodioides	—
Lastrea glanduligera	—
L. miqueliana	—
L. japonica	—
L. quelpaertensis	—
L. laxa	—
L. oligophlebia	—
Leptogramma mollissima	—
Gymnocarpium oyamense	—
Cyclosorus acuminatus	—
Abacopteris triphylla	—
Dictyocline griffithii	—
Cystopteris sudetica	—
Athyrium deltoidofrons	—
A. vidalii	—
A. wardii	—
A. niponicum	—
A. iseanum	—
A. crenatum	—
A. japonicum	—

A. conilii	−
Cornopteris decurrenti-alata	−
Diplazium subsinuatum	−
D. mettenianum	−
D. nipponicum	−
Diplaziopsis cavaleriana	
Blechnaceae	
Blechnum orientale	−
B. brasiliense	−
Struthiopteris niponica	−
Woodwardia orientalis	−
Aspleniaceae	
Asplenium antiquum	−
A. normale	−
A. trichomanes	−
A. incisum	−
A. wrightii	−
A. prolongatum	−
A. salerii	−
A. capillipes	−
Phyllitis scolopendrium	−
Camptosorus sibiricus	−
Cheiropleuriaceae	
Cheiropleuria bicuspis	−
Polypodiaceae	
Polypodium formosanum	−
P. niponicum	−
Lepisorus uchiyamae	−
L. ussuriensis	−
L. thunbergianus	−
Drymotaenium miyoshianum	−
Lemmaphyllum microphyllum	−
Neocheiropteris ensata	−
N. subhastata	−
Phlebodium aureum	−
Pyrrosia linearifolia	−
P. lingua	−
P. tricuspis	−
P. polydactylis	−
Microsorium buergerianum	−
M. normale	−
Colysis wrightii	+
C. shintenensis	+
C. pothifolia	+
C. elliptica	+
Crypsinus engleri	−
C. hastatus	−
Loxogramme saziran	−
L. salicifolia	−
L. grammitoides	−
Grammitidaceae	
Grammitis dorsipila	−
Xiphopteris okuboi	−
Ctenopteris sakaguchiana	−
Vittariaceae	
Antrophyum obovatum	−
Vittaria flexuosa	−
Marsileaceae	
Marsilea quadrifolia	−
Salviniaceae	
Salvinia natans	−
S. cuculata	−
Azollaceae	
Azolla japonica	−

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1) Mäule, C., Fünfstücks Jahrbuch **4**: 166 (1902). 2) Siersch, E., Mikrochemie **4**: 188 (1926). 3) Gibbs, R.D., The physiology of forest trees, edited by Thimann, K.V., 269 (1957). 4) Nishio, K., Ronso (Essays and Studies by Member of Kwansei Gakuin Senior High School) **6**: 34 (1961). 5) ———, Journ. Jap. Bot. **37**: 243 (1962). 6) ———, Ronso **9**: 1 (1964). 7) Tagawa, M., Coloured illustration of the Japanese Pteridophyta (1959). 8) Copeland, E. B., Genera Filicum (1947).

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27 科 97 属 205 種のシダ植物のリグニンについて，モイレ反応陽性，あるいは不明確な陽性の細胞の有無を検した。*Selaginella* (6), *Monachosorum* (2), *Ceratopteris* (2),

Elaphoglossum (2) および *Colysis* (4) は，検せられた種〔( ) 内の数字は各属で検せられた種の数〕すべてにおいて，陽性の細胞が見出された。*Plagiogyria* では *P. euphlebia* が陽性，*P. japonica* が不明確な陽性であったが，*P. adnata*, *P. matsumureana*, *P. stenoptera* は陰性であった。また，*Dennstaedtia* では，*D. hirsuta* は陽性であったが，*D. scabra* は陰性であった。*Pteridium aquilinum* は供試 13 個体中，大部分のものは陰性であったが，若干のものが，不明確な陽性を呈する細胞をもち，*Lycopodium squarrosum* は，2 個のうち，一つは不明確な陽性，他は陰性であった。他の 89 の属のものは，すべて陰性であった。

○オオカナメモチが岡山県下に自生する（林弥栄）Yasaka HAYASHI: *Photinia serrulata* found in Okayama Prefecture

オオカナメモチは中国大陸，台湾に分布し，日本では奄美諸島中の大島と徳之島，四国愛媛県下の宇和島附近に自生することが知られている。四国宇和島附近に野生のあることは最初植木秀幹博士により認められたと聞いている。広島大学の堀川芳雄博士はヒコビヤ 4: 6 にこれらの分布図を記載されている。関東以南の暖地では庭木として稀に植栽されているが，大きな照葉は美しいものがある。普通のカナメモチより葉は大形で長楕円形，楕円形，または長倒卵形をなし，長さ 10—17 cm，幅 3—6 cm 革質で上面は無毛で光沢があり，下面にも毛なくやや蒼白色をしている。縁辺には細鋸歯または刺状鋸歯がある。冬芽は赤色をなし大きい。幼葉は帯紅色であるがのち次第に暗緑色となる。新葉の出揃ったあと旧葉の大部分は美しく紅葉し，落下する。花は白色弁で，花部に毛はない。果実は球形で紅熟する。

岡山県赤磐郡瀬戸高等学校の教諭をされている大久保一治氏から岡山県下に珍しい大葉のカナメモチが山にはえているということを聞いたのは 1 昨年であった。私ははじめ中国大陸や台湾に主として自生するオオカナメモチなどが自生するはずはなく恐らくカナメモチの奇形的に葉の大きくなったものと思い，彼地で採集した腊葉を送って下さいと依頼した。間もなく一枚の標本を送ってくれた。それは花も果実もないステリルなものであったが，冬芽や葉をみ

オオカナメモチ *Photinia serrulala* 岡山県和気郡日生町寒河産.